ART 34 AMDT

Amended claims

Claims:

1. Method for screening of modulators of calcineurin activity, characterized in that an interaction between calcineurin and superoxide dismutase is monitored, comprising the following steps
   - forming of a complex comprising at least calcineurin and superoxide dismutase under incubation with at least one potential modulator,
   - detecting the influence of the potential modulator by directly monitoring the complex formation and/or by monitoring the activity, especially the enzymatical activity of the complex.

2. Method according to claim 1, characterized in that the superoxide dismutase is a Copper/Zinc-superoxide dismutase.

a 3. Method according to claim 1 ~~or 2~~, characterized in that forming of the complex is performed in the presence of the potential modulator.

a 4. Method according to claim 1 ~~or 2~~, characterized in that the potential modulator is added after the complex has been formed.

a 5. Method according to ~~one of the preceding claims~~ claim 1, characterized in that the monitoring is performed by detection of labels, especially fluorescent labels.

a 6. Method according to ~~one of the preceding claims~~ claim 1, characterized in that calcineurin and/or superoxide dismutase

carry labels, especially fluorescent markers, wherein preferably the labels are enhanced green fluorescent protein.

7. Method according to claim 6, characterized in that calcineurin and/or superoxide dismutase are expressed as fluorescent proteins, particularly as fusion proteins together with enhanced green fluorescent protein.

a 8. Method according to Claim 1 ~~one of the preceding claims~~, characterized in that the monitoring of complex formation is performed by laser fluctuation correlation spectroscopy.

a 9. Method according to Claim 1 ~~one of the preceding claims~~, characterized in that calcineurin and superoxide dismutase are coexpressed in cells, especially in eukaryotic cells, and that the complex formation is performed within the cell.

a 10. Method according to Claim 1 ~~one of the preceding claims~~, characterized in that calcineurin and/or superoxide dismutase are expressed in cells, especially in prokaryotic cells, and that calcineurin and/or superoxide dismutase are isolated and/or purified before the complex formation is performed.

11. Method according to claim 10, characterized in that purification of calcineurin is achieved by affinity chromatography, especially by ferro-nitrilotriacetat-metal affinity chromatography.

12. Method according to claim 10, characterized in that purification of superoxide dismutase is achieved by

affinity chromatography, especially by copper/zinc-nitrilotriacetat-metal affinity chromatography.

13. Method according to ~~one of the preceding claims~~ Claim 1, characterized in that in the complex formation step additionally calmodulin and/or calcium is added.

14. Method according to ~~one of the preceding claims~~ Claim 1, characterized in that the monitoring of the enzymatical activity is performed by analyzing the phosphatase activity of calcineurin.

15. Method according to claim 14, characterized in that the phosphatase activity is analyzed by the use of at least one substrate, which preferably carries a label, especially a fluorescent label.

16. Method according to claim 15, characterized in that the substrate is a peptide, especially a peptide characterized by the amino acid sequence

Asp - Leu - Asp - Val - Pro - Ile - Pro - Gly - Arg - Phe - Asp - Arg - Arg - Val - Ser - Val - Ala - Ala - Glu.

17. Method according to claim 15 ~~or 16~~, characterized in that the substrate is a peptide containing a residue, especially a serine residue, labeled with fluoresceine.

18. Method according to ~~one of claims 3 to 17~~ Claim 3, characterized in that prior or after detecting the influence of the potential modulator on the complex formation and/or complex activity the influence of the potential modulator on the activity, especially the enzymatical activity of calcineurin is detected seperately.

19. Method for screening of modulators of calcineurin activity, especially according to ~~one of the preceding claims~~ claim 1, comprising

a) determining the interaction of a potential modulator with either calcineurin or superoxide dismutase as a partner,

b) taking a potential modulator showing interaction with calcineurin or superoxide dismutase according to step a),

c) determining the interaction of said modulator taken in step b), with the other partner, namely calcineurin or superoxide dismutase, respectively, and

d) identifying the potential modulator showing interaction also according to step c).

20. Method according to claim 19, characterized in that calcineurin and/or superoxide dismutase comprises at least one tag, especially a histidine tag.

21. Method according to claim 19 ~~or claim 20~~, characterized in that said superoxide dismutase is a Copper/Zinc-superoxide dismutase.

22. Method according to ~~one of claims 19 to 21~~ claim 19, characterized in that calcineurin and/or superoxide dismutase is attached to a solid matrix, especially a Ni-NTA, Fe-NTA and/or CuZn-NTA matrix.

23. Kit for screening of modulators of calcineurin activity comprising

- calcineurin and/or a vector encoding for calci-

neurin and/or cells capable of expressing calcineurin, and

- superoxide dismutase and/or a vector encoding for superoxide dismutase and/or cells capable of expressing superoxide dismutase.

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